

CLAIMS

1. A method for sequencing a polynucleotide, comprising the steps of:

5 (i) reacting a target polynucleotide with a polymerase enzyme immobilised on a solid support, and the different nucleotides, under conditions sufficient for the polymerase reaction; and

10 (ii) detecting an effect consequent on the incorporation of a specific nucleotide complementary to the target polynucleotide.

~~2. A method according to claim 1, wherein the effect in step (ii) is detected by measuring radiation.~~

~~3. A method according to claim 1 or claim 2, wherein steps (i) and (ii) are conducted with each of the different nucleotides in turn, until incorporation is detected, and then repeated.~~

4. A method according to claim 1 or claim 2, wherein step (i) is conducted with all the nucleotides present.

20 5. A method according to ^{claim 1} any preceding claim, wherein the nucleotides comprise a 3' blocking group which is removed after the polymerase reaction.

6. A method according to claim 5, wherein the blocking group can be selectively removed by pulsed monochromatic light

25 7. A method according to claim 5 or claim 6, wherein the nucleotides comprise a further blocking group at the terminal phosphate group of the triphosphate chain, and the further blocking group is removed prior to the removal of the 3' blocking group.

30 8. A method according to claim 7, wherein the further blocking group can be selectively removed by pulsed monochromatic light under conditions different from those required to remove the 3' blocking group.

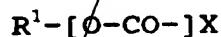
35 9. A method according to claim 8, wherein the further blocking group is removed by pulsing the monochromatic

light for a duration different from that required to remove the 3' blocking group.

- a 10. ^{The} A method according to ^{claim 1} ~~any preceding claim~~, wherein step (i) further comprises introducing a competitive inhibitor of the polymerase enzyme.
- a 11. ^{The} A method according to ^{claim 1} ~~any preceding claim~~, wherein the target polynucleotide of step (i) is bound to the polymerase enzyme by a β_2 dimer complex.
- a 12. ^{The} A method according to ~~any preceding claim~~, wherein the polymerase is E. coli DNA polymerase III or T7 polymerase.
- a 13. ^{The} A method according to ^{claim 1} ~~any of claims 1 to 11~~, wherein the polymerase is Taq polymerase.
- a 14. ^{The} A method according to ^{claim 1} ~~any of claims 1 to 11~~, wherein the polymerase is reverse transcriptase.
- a 15. ^{The} A method according to ^{claim 1} ~~any preceding claim~~, wherein step (ii) comprises detection of a change in resonance signal over time.
- a 16. ^{The} A method according to ^{claim 1} ~~any preceding claim~~, wherein the radiation is electromagnetic.
- a 17. ^{The} A method according to claim 16, wherein the electromagnetic radiation is in the infra-red spectrum.
- a 18. ^{The} A method according to ^{claim 1} ~~any preceding claim~~, wherein step (ii) comprises using surface plasmon resonance.
- a 19. ^{The} A method according to claim 16, wherein the electromagnetic radiation is in the radio-frequency spectrum.
- a 20. ^{The} ~~A method according to claim 19, wherein the incorporation of a nucleotide is detected using NMR.~~
- a 21. ^{The} A method according to ^{claim 1} ~~any preceding claim~~, wherein the polynucleotide is DNA.
- a 22. A sensor chip comprising a polymerase enzyme immobilised thereon.
- a 23. A nucleotide comprising a blocking group at the 3' position and at the terminal phosphate group of the triphosphate chain, wherein the two blocking groups are removable by monochromatic light of different wavelengths.

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a 24. ^{The} ~~A~~ nucleotide according to claim 23, wherein the blocking groups are derived from a compound of the formula



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wherein R^1 is a photolabile group and X is a leaving group.

a 25. ^{The} ~~A~~ nucleotide according to claim 23 ~~or claim 24~~, wherein the blocking group at the 3' position is an o-nitrobenzyloxycarbonyl group.

a 10

26. ^{The} ~~A~~ nucleotide according to ^{claim 23} ~~any of claims 23 to 25~~, wherein the blocking group at the terminal phosphate is an o-nitrobenzyl group.

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a 27. ^{The} ~~A~~ nucleotide according to ^{claim 23} ~~any of claims 23 to 26~~, wherein the blocking group at the 3' position is a (4,5-dimethoxy-2-nitrobenzyl)oxycarbonyl group.

a 28. ^{The} ~~A~~ nucleotide according to ^{claim 23} ~~any of claims 23 to 27~~, wherein the blocking group at the terminal phosphate is a 1-(2-nitrophenyl)ethyl group.

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29. An apparatus for sequencing a polynucleotide, comprising an optical sensor chip, a light source, an imaging device and a photodetector, wherein the sensor chip is as defined in claim 22.

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